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Temporal and Spatial Variation of Fungal Concentrations in Indoor Air

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The aim of this study was to determine temporal and spatial variation of airborne concentrations of viable fungi in 2 single-family houses during the period of the year that such levels are mostly determined by indoor sources. One of the subject residences had moisture problems (the index residence), the other did not have any moisture problems (the reference residence). The concentrations of viable fungi in indoor air were determined a total of 6 times during the winter with 2 six-stage impactors (Andersen 10-800) in 2 rooms in both of the houses. The total concentrations of viable fungi and concentrations of *Penicillium, Aspergillus*, and *Aspergillus versicolor* were significantly higher in the index residence than in the reference residence. Statistically significant differences in the total concentrations were observed in the size ranges of 2.1–3.3 μm and 1.1–2.1 μm. In both residences, there was a statistically significant difference between the rooms in the total concentrations of viable fungi, although in the reference residence that was not of practical value because the levels were low. In concentrations of fungal genera, a significant difference, that was also of practical importance, was seen in the concentrations of *Penicillium* in the index residence. Both within-day and within-season differences of concentrations were observed in the residences: a trend of higher values in the morning and at the beginning of winter was noted. In order to characterize fungal concentrations of a residence with unknown indoor conditions, a sampling campaign of 11 different days is needed. A strategy for responding to an alert or alarm situation is presented.

INTRODUCTION

Sampling of airborne viable fungi is generally used to determine fungal contamination of buildings, even though it is not recognized as an infallible method for that purpose (Dillon et al. 1996). Filter sampling for determination of the total number of fungal particles is recommended in parallel with sampling of viable fungi (Samson et al. 1994). However, in home environments the total number of fungi is usually lower than the relatively high detection limit of this sampling method (Hyvärinen et al. 1996; Rautiala et al. 1996). Airborne concentrations of viable fungi may be slightly elevated due to microbial growth in moistened material (Hunter et al. 1988; Hyvärinen et al. 1993; Hyvärinen et al. 1999). In addition, differences in the flora are seen between moldy and reference buildings (Pasanen et al. 1992; Hyvärinen et al. 1993). No one nutrient medium covers the entire range of airborne fungi, but the parallel use of 2 nonselective media, 2% malt extract agar and dichloran glyserol agar (DG18), is recommended for detection of viable fungi (Samson et al. 1994).

The most typical genera found in indoor air are *Penicillium*, *Aspergillus*, and *Cladosporium* (Hunter et al. 1988; Pasanen et al. 1992; Hyvärinen et al. 1993). Certain fungi, such as *Stachybotrys*, *Fusarium*, *Trichoderma*, and *Aspergillus versicolor*, are regarded as indicators of moisture problems if found in air samples (Samson et al. 1994). Yet total concentrations of viable fungi observed in moldy buildings are not necessarily higher than those observed in control houses with no evidence of mold (Strachan et al. 1990; Flannigan et al. 1991; Nevalainen et al. 1991). The range of indoor air concentrations of airborne viable fungi is wide, $10–10^4$ cfu/m^3, and airborne concentrations of fungi vary both temporally and spatially (Hunter et al. 1988; Verhoeff et al. 1990; Pasanen et al. 1992), although the quantity and causes of such variation are not well understood. One of the causes of variation is the variety of sources and their mode of operation. The main source of airborne fungi in indoor air is usually outdoor air (Flannigan et al. 1991; Levetin 1995). In subarctic climate, however, the outdoor air levels of microbes are extremely low in winter due to frozen soil and snow cover and the contribution of outdoor air to indoor air microbial levels is negligible (Reponen et al. 1992). Therefore, to identify indoor sources of microbes and measure the resulting levels, sampling is preferably performed during the winter months. Other common sources of airborne fungi can be everyday activities

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releasing fungi, such as handling organic materials, resuspension of spores as a result of cleaning activities, transport of spores in clothes and pets (Lehtonen et al. 1993), and, finally, mold growth in building structures (Hunter et al. 1988), the detection of which is most often the reason for indoor air sampling. The release of spores from moldy structures is not continuous and is dependent on ambient conditions such as air velocity and relative humidity (Pasanen et al. 1991). Spores are released more easily into dry air (Zoberi et al. 1961), although the relative humidity of the air does not directly influence fungal growth on building materials (Pasanen et al. 1991).

The underlying but rarely stated assumption in many epidemiological studies is that fungal contamination in a residence is homogeneous over space and time. Because of limited funds and resources available for any particular investigation, only a few samples may be taken from each studied building and conclusions of the contamination level are reached based on those (few) samples. However, it is not known how well one or a few samples of airborne viable fungi represent the fungal contamination of a residence. Therefore the objectives of this study are to examine whether one or a few measurements at one site over a season is sufficient to characterize fungal levels in a residence and to study if one measurement day is sufficient to characterize the levels in a residence for the winter season. If one measurement site and day is not sufficient, an additional objective of this study is to recommend how many samples are required to characterize the fungal concentration of a residence and to relate this sample size with the guidelines presently used for diagnosing residences with problems associated with fungal contamination (Ministry of Social and Health Affairs 1997). A secondary objective of this study is to examine whether differences in fungal concentrations, if any, are dependent on the 2 growth media used.

MATERIAL AND METHODS

This study is not a survey study, rather it is a case study that investigates temporal and spatial variation of fungal contamination in 2 residences to provide the background for an experimental design for a projected survey study. Several residences were inspected for moisture problems and visible microbial growth by trained civil engineers. The subject residences were selected from each of their respective categories. One of the subject residences, a single-family house, belongs to a class of homes in which engineers observed moisture problems. The other subject residence, also a single-family house, was selected from among houses with no observed moisture problems. In the balance of this paper, the former house is called the "index residence" and the latter the "reference residence." The houses were matched for building type, age, site, use, occupancy, and architecture.

Indoor fungal contamination during the months between April and September are mainly driven by outdoor levels (Reponen et al. 1992). However, during the period between October and March the ground is usually covered by snow in central Finland and any indoor variation is due to indoor sources (Reponen et al. 1992). To study temporal and spatial variation in the 2 residences, 2 sites were sampled in each residence, which were typical sized single-family houses (about 120 m^2). One site was the bedroom that was less occupied during the period of sampling, the other was the dining room where most of the indoor activity took place during sampling times. To ensure efficient generation of biocontamination, both houses selected were daycare homes with children visiting over the sampling period.

Air samples were taken in 2 rooms in both houses every other week from October to February, for a total of 6 times during the sampling period. Outdoor air samples were taken once each sampling day. Samples were taken with six-stage impactors (Andersen 10-800) to determine the concentrations and the composition of the flora of viable fungi.

During each sampling day 6 impactor samples were taken, 3 in a dining room and 3 in a bedroom, in 3 2-hour sampling periods. Each sampling period started at about 9:00 am, 11:00 am, and 1:00 pm. The sampling time was 10 min. Samples were taken at the middle of the rooms, at a height of 1–1.5 m representing the breathing level of a sitting adult person or a standing child. Occupants were asked to avoid activities, such as vacuuming or handling unwashed vegetables, before and during the sampling.

The impactor samples were taken at a flow rate of 28.3 L/min. Two samples were taken simultaneously, each was collected on a different growth medium of 2% malt-extract agar (MEA) and dichloran glycerol agar (DG18). Fungi were incubated in the dark at 25°C for 5–7 days. Fungal colonies were identified by genus using an optical microscope. In addition, the species of the *Aspergillus versicolor* group were separately identified. Concentrations (cfu/m^3) were calculated using flow rate, sampling time, and the Andersen correction table for multiple impactions on individual sites (Andersen 1958).

Statistical methods used were Pearson's correlation coefficient, paired and 2 samples t-test, analysis of variance (ANOVA), Wilcoxon's rank test, and Wilcoxon's signed rank test. The differences were considered to be statistically significant with $\alpha = 0.05$.

Even though a number of genera were identified, the ones tested in the statistical analysis were *Penicillium*, *Aspergillus*, *Cladosporium*, and yeasts, which are the most typically found genera or group (yeasts) in indoor air in Finland (Pasanen et al. 1992; Hyvärinen et al. 1993) and *Aspergillus versicolor*, which represents indicator microbes (Samson et al. 1994).

RESULTS

Exploratory data analyses revealed that the distributions of the data obtained in this study were not normal and therefore geometric means were used in the tables to describe the data. A logarithmic transformation was performed on the original data: the distributions of log-transformed concentrations were normal for total viable fungi and *Penicillium*, but not for the other genera. An extreme value was identified in the fungal data: its magnitude was nearly 8 times as high as the second highest observed value. Several statistical tests identified this value as the only outlier of the database. Analyses of the fungal data

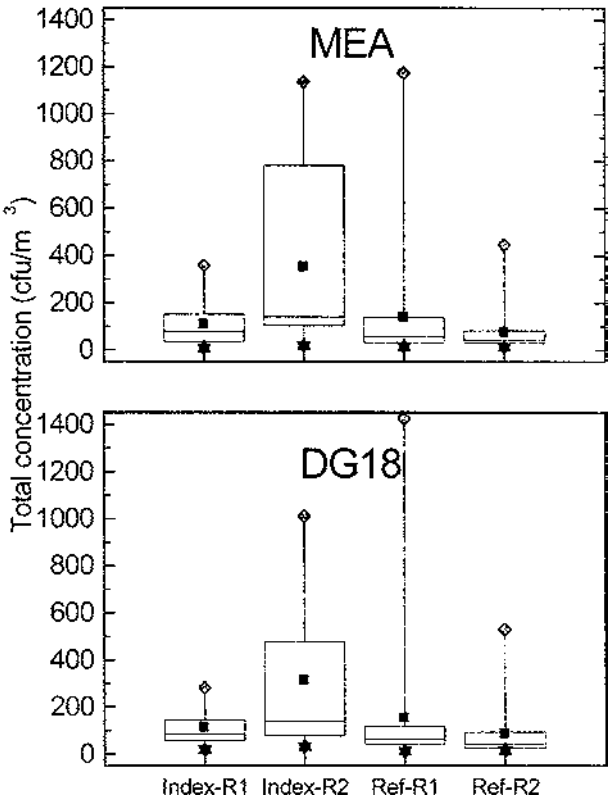

Figure 1. Distributions of fungal concentrations in two rooms (R1, R2) of the index and reference residences. Distributions are presented in 2 media (MEA, DG18).

were performed excluding this extreme value. Concentrations of different individual genera that were below the detection level (4 cfu/m^3) were treated in the analyses as zeros. The percentage of values below detection levels for individual genera varied from 0.7% for *Penicillium* to 76.8% for *Aspergillus versicolor*. The distributions of the data are shown in Figure 1.

The geometric means, geometric standard deviations, and ranges of the concentrations of airborne viable fungi in the 2 media and residences are presented in Table 1. The geometric means of the total concentrations of viable fungi in outdoor air were approximately 25 cfu/m^3. Therefore it was concluded that outdoor air had only a negligible contribution to fungal levels indoors.

Differences Between the Growth Media

The fungi were sampled on 2 growth media, MEA and DG18, because a greater number of genera is expected by using these 2 media than by using either medium separately. The correlation between the 2 media was high for total concentration ($r = 0.92$, $p = 0.0001$) and concentrations of *Penicillium* ($r = 0.91$, $p = 0.0001$), *Aspergillus* ($r = 0.74$, $p = 0.0001$), and *Aspergillus versicolor* ($r = 0.67$, $p = 0.0001$). Concentrations of yeasts and *Cladosporium* did not correlate as well with $r = 0.29$ ($p = 0.0152$) and $r = 0.30$ ($p = 0.0104$), respectively. Yet, 61% of the concentrations of *Cladosporium* was under the detection limit (4 cfu/m^3) and the concentrations detected were very low, <28 cfu/m^3. Therefore it was concluded that for application purposes the 2 media led to equivalent *Cladosporium* levels.

Table 1
GM, GSD, and range of the total concentrations of viable fungi and concentrations of different mold genera or yeasts in the residences (index, reference) and in 2 media (MEA, DG18)

	Index			Reference		
	GM	GSD	Range	GM	GSD	Range
MEA ($n = 36$)						
Total	116	3.3	7–1136	57	2.7	11–1175
Penicillium	61	4.6	0–1114	33	3.4	4–1149
Aspergillus	8	5.2	0–407	0.4	2.0	0–7
Cladosporium	1	2.4	0–12	0.9	2.5	0–14
Yeasts	5	3.4	0–34	8	3.2	0–50
A. versicolor	2	4.3	0–78	—	1.0	0–0
DG18 ($n = 35/36$)						
Total	122	2.8	18–1011	61	2.7	11–1425
Penicillium	72	3.5	7–991	41	3.0	7–1398
Aspergillus	10	3.5	0–124	0.6	2.2	0–11
Cladosporium	2	3.2	0–28	1	2.8	0–14
Yeasts	4	3.4	0–25	8	2.6	0–27
A. versicolor	3	4.4	0–51	—	1.0	0–0

On the other hand, concentrations of yeasts were mostly (80%) above the detection limit; therefore it was concluded that these concentrations differed depending on the growth media used. The difference in the total concentrations between the 2 media was also tested with a paired t-test; the null hypothesis was rejected at a significance level of $\alpha = 0.05$. However, the p-value (0.0397) was in a borderline region and therefore the data were examined to determine if the statistically significant difference was of practical importance. Inspection of the raw data led to the conclusion that, from a practical point of view, the 2 media led to equivalent concentrations. In the following analysis of the total concentrations of viable fungi and the concentrations of *Penicillium*, *Aspergillus*, and *Cladosporium*, the results of the 2 growth media were considered as 2 independent values for analysis. The analysis of yeasts was performed separately for each medium.

Differences Between Residences

The 2 residences studied were characterized as index and reference based on the observations of moisture damage in the residences as described in the material and methods section. The null hypothesis of no difference between the 2 residences in the total concentrations of viable fungi was tested with a t-test against the alternate hypothesis that the index residence has higher concentrations of fungi. It was concluded that the difference is statistically significant and that the total concentrations of viable fungi in the index house were higher than the concentrations in the reference house ($p = 0.0001$). In addition, the null hypothesis of no difference between the residences in median concentrations of each size class was rejected (Wilcoxon Rank test) in size ranges of 2.1–3.3 μm and 1.1–2.1 μm. The concentrations in these size ranges were significantly higher in the index house than those in the reference house ($p < 0.0001$; Figure 2). No differences were observed in the size distributions of outdoor air near the index and reference residences.

Since the distributions of most genera were not normal (even when log-transformed), the homogeneity of the levels of different genera between the residences was tested with a nonparametric Wilcoxon rank test. The null hypothesis of no difference in the median of the fungal concentrations between the residences was tested. It was rejected in the concentrations of *Penicillium* ($p = 0.0025$) and *Aspergillus* ($p = 0.0001$); hence, it was concluded that the concentrations of *Penicillium* and *Aspergillus* were significantly higher in the index house than in the reference house. A similar test indicated that we failed to reject the null hypothesis of no difference between the residences for the concentrations of *Cladosporium* and the yeasts. Thus the concentrations of *Cladosporium* and yeasts were at the same level in both houses. *Aspergillus versicolor* was found only in the index house.

Differences Between the Rooms

The geometric means, geometric standard deviations, and ranges of the concentrations of airborne viable fungi in the 2 rooms are presented in Table 2. Results of the 2 residences and 2 media are presented separately.

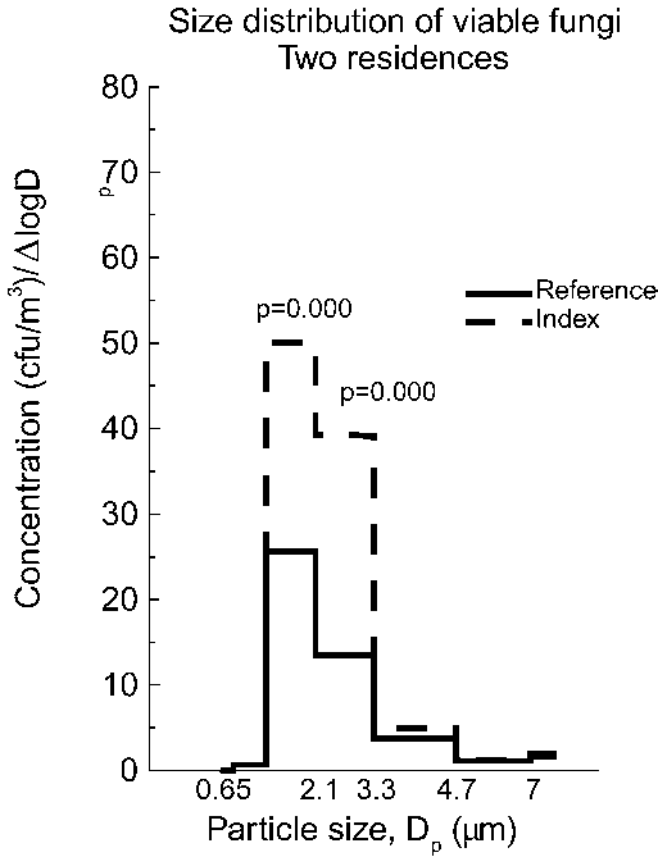

Figure 2. Size distributions of airborne viable fungi in the index and the reference residences.

Paired t-tests were used to investigate the difference in the total concentrations of viable fungi between the 2 rooms. The difference in fungal levels was tested using the difference, δ, of corresponding values, H_0: $\delta = 0$. Because the test used the difference between simultaneous room measurements (Room 1–Room 2), analyses could be done by merging the data from both of the residences even though the residences differed in fungal levels. For both residences, Room 1 was considered to be the room with lower average concentration of total viable fungi. The null hypothesis was rejected with p-value $= 0.0001$; hence, the difference in total concentrations of fungi between the 2 rooms was statistically significant. This was supported by the analysis that treated the residences separately (Index: $p = 0.0001$; Reference: $p = 0.0014$). The statistically significant difference in concentrations of total viable fungi between the rooms in the reference residence was not of practical value because the levels were frequently very low. This was not the case in the index residence, where the concentration difference between the rooms was also of practical importance.

The concentration difference between the rooms of the index residence was also seen in 4 size ranges (4.7–7 μm ($p = 0.004$), 3.3–4.7 μm ($p = 0.001$), 2.1–3.3 μm ($p = 0.013$), 1.1–2.1 μm ($p = 0.036$); Wilcoxon's rank test; Figure 3a). In room 1 of

Table 2
GM, GSD, and range of the total concentrations of viable fungi and concentrations of different mold genera or yeasts in 2 rooms (R1, R2); the results of the residences (index, reference) and 2 media (MEA, DG18) are presented separately

	Index ($n = 18$)				Reference ($n = 18$)			
	GM (GSD)		Range		GM (GSD)		Range	
	R1	R2	R1	R2	R1	R2	R1	R2
MEA								
Total	73 (2.7)	184 (3.3)	7–357	18–1136	67 (3.0)	48 (2.5)	14–1175	11–446
Penicillium	32 (4.2)	116 (4.0)	0–217	11–1114	33 (4.2)	33 (2.8)	4–1149	4–443
Aspergillus	8 (4.6)	9 (6.1)	0–150	0–407	0.7 (2.2)	0.2 (1.6)	0–7	0–4
Cladosporium	0.8 (2.3)	0.7 (2.6)	0–7	0–12	1 (2.6)	0.4 (2.3)	0–14	0–11
Yeasts	6 (3.2)	5 (3.6)	0–34	0–25	14 (2.4)	5 (3.5)	0–50	0–36
A.versicolor	3 (3.9)	2 (5.0)	0–78	0–66	— (—)	— (—)	0–0	0–0
DG18								
Total	86 (2.2)	172 (3.0)	18–282	32–1011	73 (2.8)	51 (2.6)	11–1425	14–529
Penicillium	47 (2.8)	111 (3.8)	7–267	18–991	46 (3.3)	36 (2.7)	7–1398	7–525
Aspergillus	11 (3.2)	9 (4.0)	0–124	0–92	0.8 (2.3)	0.5 (2.1)	0–11	0–7
Cladosporium	2 (3.4)	2 (3.1)	0–22	0–28	1 (2.9)	2 (2.7)	0–14	0–11
Yeasts	3 (3.8)	6 (3.0)	0–25	0–25	11 (2.2)	6 (2.8)	0–27	0–26
A.versicolor	2 (4.5)	4 (4.3)	0–51	0–44	— (—)	— (—)	0–0	0–0

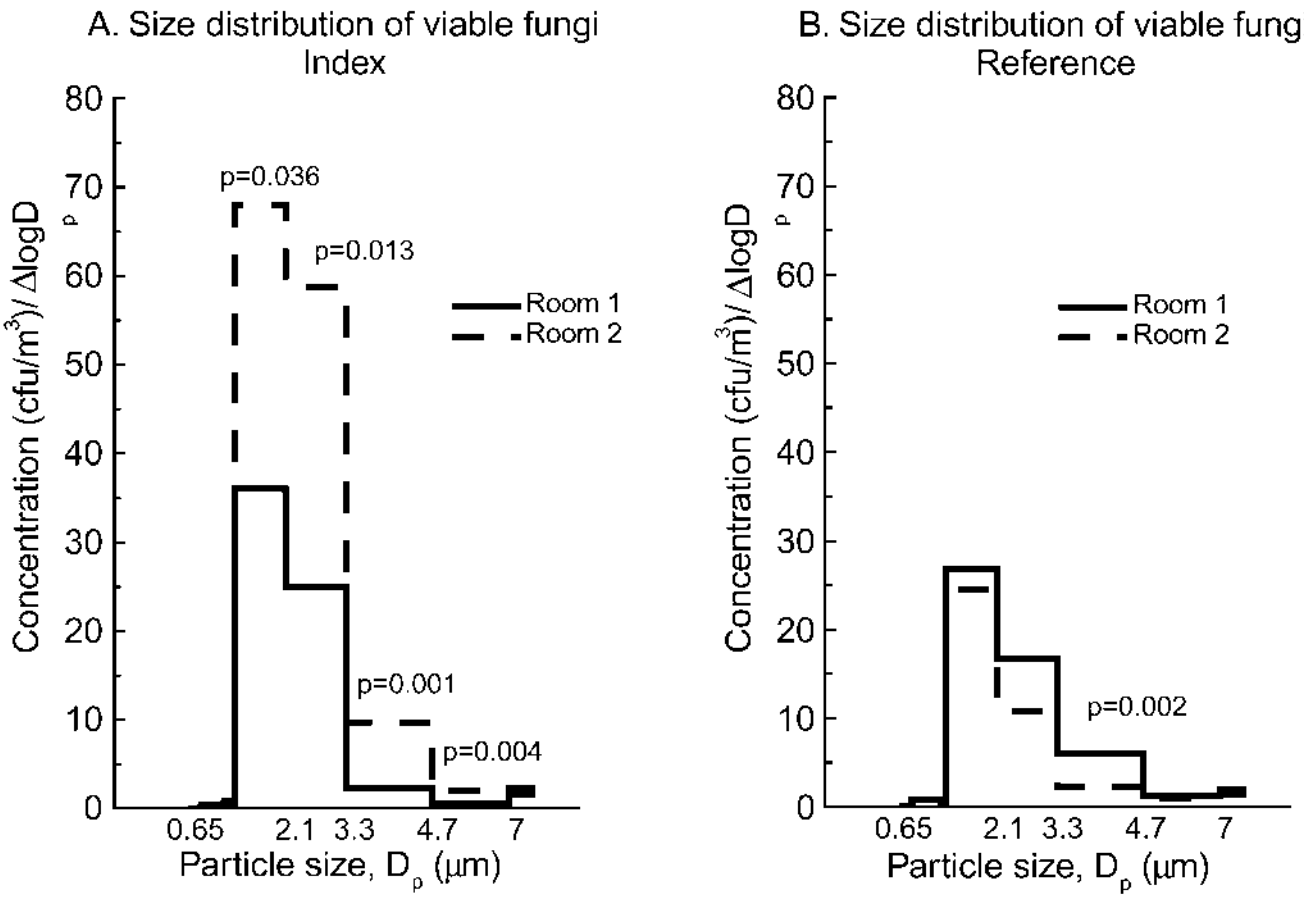

Figure 3. Size distributions of airborne viable fungi in the 2 rooms of the index and the reference residences.

the reference residence, significantly higher concentrations were observed in size range 3.3–4.7 μm ($p = 0.002$), but that has little practical importance because of the low levels (Figure 3b).

A nonparametric Wilcoxon signed rank test was used to test the null hypothesis of no difference in the median of the concentrations of different genera between the rooms. The null hypothesis could not be rejected for *Aspergillus*, *Aspergillus versicolor*, and *Cladosporium*, but it was rejected in the concentrations of *Penicillium* and yeasts. The difference in concentrations of *Penicillium* between the rooms was significant ($p = 0.0001$). The difference in room concentrations of yeasts sampled using MEA was significant ($p = 0.022$), whereas the difference in room concentrations observed with DG18 growth medium was not ($p = 0.073$).

When the residences were processed separately, the null hypothesis of no difference in the median of different genera between the rooms could not be rejected for concentrations of *Aspergillus*, *Aspergillus versicolor*, and *Cladosporium*, which supported the analysis done by merging the data from the residences. The null hypothesis was rejected for concentrations of *Penicillium* ($p = 0.0001$) in the index residence, which was in agreement with the analysis of the merged data from both of the residences. In the reference residence, however, the difference was not statistically significant. For concentrations of yeasts, the difference between the rooms was not statistically significant in the index residence in both of the growth media, whereas it was significant in the reference residence (MEA: $p = 0.002$; DG18: $p = 0.033$). The concentrations of the yeasts varied from <4 cfu/m^3 to 50 cfu/m^3. More than 90% of the difference between the rooms were <20 cfu/m^3 and therefore the difference was not of practical importance.

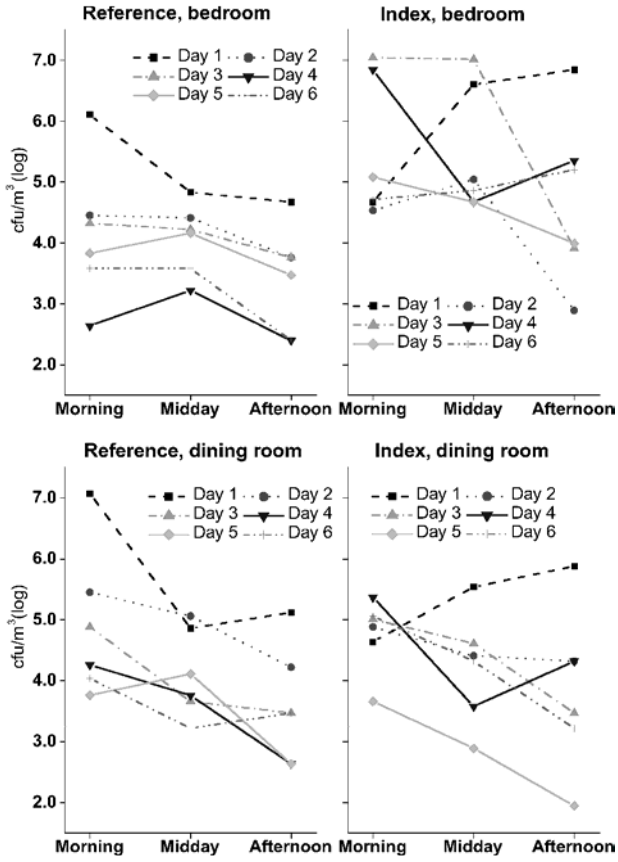

Figure 4. Within-day and within-season variation of fungal concentration (MEA) in 2 rooms in the index and reference residences.

Temporal Variation

A 2-way Anova was used to test for within-day and within-season variation. In the absence of such variations, no repeated measurements would be required to characterize fungal levels in each residence. There were no interaction effects. In the index residence, both within-day and within-season differences of concentrations in the dining room were statistically significant with $p = 0.0025$ and $p = 0.001$, respectively. In the bedroom, the within-day difference was marginally significant ($p = 0.0475$), but the within-season difference was marginally not significant ($p = 0.0665$). All temporal differences in the reference residence were statistically significant ($p = 0.0001$).

In the index and reference residences, total concentrations of fungi were usually higher in the morning than in the afternoon (Figure 4). The concentrations observed in November and in the beginning of December (days 1–3), especially in the reference residence, were mostly higher than those observed in February (days 5–6; Figure 4).

Suggested Method For Characterizing Fungal Contamination During The Winter Months

Since one sample per residence or per day and per season is not sufficient to characterize fungal contamination of a residence, the number of samples required for this purpose must be estimated. Sample size relates to the precision of measurement, yet the largest sample size is not necessarily the best sample size; cost and time must be considered. The sample size to be determined must be both sufficient and efficient. The margin of error, an indicator of the precision of the estimate, is defined as one-half the width of the confidence interval of the mean. The margin of error, E, the significance level, $\alpha = 0.05$, and an estimate of the population standard deviation are combined to estimate a sufficient and efficient sample size using the following equation: $n = (Z_{\alpha/2})^2\sigma^2/E^2$ (Freund and Wilson 1997).

The margin of error was calculated using the log-transformed sample database of the index residence, $E = 0.785$. The population standard deviation was obtained by dividing the population range by 4. Several databases were merged to determine the population range of index residences in Finland (Hyvärinen et al. 1993; Hyvärinen et al. 1996; Rautiala et al. 1996; Hyvärinen et al. 1999; and others). The range between 5th and 95th percentile values of the merged database of fungal concentrations, $n = 129$, was used to estimate the population standard deviation. The estimated value of σ was 1.32. The sample size required to characterize the fungal concentration of an index

residence is $n = 11$. The sample size was calculated that way so that the true parent mean lies inside the 95% confidence interval.

DISCUSSION

Total mean concentrations of viable fungi were higher in the index residence than those in the reference residence, though they varied almost in the same range. This agrees with some earlier studies done in Finland (Hyvärinen et al. 1993; Hyvärinen et al. 1999). However, other studies assert that concentrations are not always higher in buildings with moisture problems compared to those in reference buildings (Strachan et al. 1990; Flannigan et al. 1991; Nevalainen et al. 1991). Concentrations of *Penicillium* and *Aspergillus* were higher in the index residence than in the reference residence, and *Aspergillus versicolor* was found only in the index residence. Therefore the statement that fungal composition of air samples is different in index buildings than in reference buildings (Pasanen et al. 1992; Hyvärinen et al. 1993) was also supported by this study. The difference in the concentrations between the residences was largest in the size ranges 2.1–3.3 μm and 1.1–2.1 μm. This finding was in accordance with an earlier study for the size range 2.1–3.3 μm (Reponen et al. 1994).

In both residences, the most prevalent and abundant fungal genus was *Penicillium*. *Penicillium* species are the most typical genera in indoor air. They produce large numbers of dry and small spores (Miller 1992), which are also easily released from normal sources and everyday activities, such as handling of foodstuff and pets and their bedding (Hunter et al. 1988; Lehtonen et al. 1993).

In this study, the variation of fungal concentrations in the buildings is considered to be only due to indoor sources, since the study was conducted during winter when the contribution of outdoor air to indoor air microbial levels is negligible (Reponen et al. 1992). In the index residence, the concentrations of viable fungi were higher in the bedroom than in the dining room. This may be due to differences in the mold sources and/or differences in the activities in the rooms. In the reference residence, no difference of practical value was observed between the rooms. This supports the idea that the difference in the index residence was due to seriousness of mold damage in rooms because the activity was considered to be similar in both of the residences. The release of spores from moldy surfaces or structures depends on ambient conditions (Pasanen et al. 1991) from which air velocity is connected with the activities in rooms and relative humidity and temperature are at the same level throughout accustomed-sized residences. Concentration variations due to transport of spores through clothes or pets (Lehtonen et al. 1994) were also considered to be minimal in this case because the occupants of neither house had attended places (barns, gardens, etc.) from where spores could be transported into the residences, and during winter months transport through cat's fur (the index residence) is unlikely. Ventilation effects fungal concentrations mainly during nonwinter months via transport of spores from outdoors (Reponen et al. 1989), but especially if the air supply is not controlled, airflows due to ventilation may affect concentration variation by moving thriving spores from moldy structures into the air.

The results of size distributions affirm the difference between the rooms observed in the index residence since concentrations in the other room of the index residence were significantly higher in 4 size ranges, whereas in the reference residence, that was the case only in 1 size range.

The determination of the concentrations of airborne viable fungi is affected by activities, sources, accuracy of the sampler, growth medium used, viability of spores, etc. (Miller 1992; Nevalainen et al. 1992). While others have indicated that indoor fungal concentrations vary in time and space (Hunter et al. 1988; Verhoeff et al. 1990; Pasanen et al. 1992), this study demonstrates the variation within day and season and between rooms. In the reference residence, however, with overall low fungal levels, relatively small changes in concentrations appeared to influence statistical significance. Total concentrations were usually higher in the morning than in the afternoon. This may reflect the activity peak in the mornings when adults go to work, school-age children go to school, and day-care children arrive and go out for play. A trend of higher concentrations in the beginning of the winter season was shown, and will be considered in future studies.

The 2 growth media used in this study gave similar results for the fungal concentrations. Only concentrations of yeasts displayed differences of practical importance. However, this conclusion may not hold true for all genera.

Due to the large variation of fungal concentration within space and time, a sampling campaign of 11 different days is needed to characterize the airborne fungal concentrations of a residence with unknown indoor conditions in subartic climate. Within-day variation is addressed by sampling at different times in each of the 11 sampling days. Within-space variation is addressed by sampling 2 rooms of a subject residence during each sampling day. This approach will be adjusted in determining potential contamination in residences where people are having symptoms indicating mold problems and where no visible signs of moisture or mold problems are seen. Visible signs of mold in nonindustrial environments are conclusive indicators of health risks (WHO 1990; Samson et al. 1994). Therefore residences with such signs do not require air sampling, they require steps to control the problem.

The pertinent guidelines in Finland (Ministry of Social Affairs and Health 1997) state that (1) fungal concentrations above 100 cfu/m^3 and the presence of indicator microbes are an alert indicating a possible abnormal indoor source of fungi. In this case no more air sampling is needed and engineering investigations should commence to identify and control the potential source. (2) Concentrations above 500 cfu/m^3 constitute an alarm and the process is the same as in (1). This study suggests that if neither of the above scenarios occurs, the sampling of indoor fungi must continue at least for 6 times over 2 months in the

subject residence. It is further suggested that if the coefficient of variation is <20%, there will be no need for any additional samples; the mean fungal contamination of the subject residence is thought to be stable. If the coefficient of variation is larger than 20%, the remaining 5 samples should be taken over the next 2 month period to fully characterize fungal contamination of the residence.

The objective of the Finnish effort is to determine associations between indoor air conditions and symptoms and to design control strategies that will reduce the frequency and intensity of the symptoms. The approach outlined in this paper combines both statistical and practical considerations to identify and eventually control sources of fungal contamination, the ultimate goal of this microbial research program.

REFERENCES

Andersen, A. A. (1958). New Sampler for the Collection, Sizing and Enumeration of Viable Airborne Particles, *J. Bacteriology* 76:471–484.

Dillon, K., Heinsohn, P., and Miller, D., editors (1996). *Field Guide for the Determination of Biological Contaminants in Environmental Samples*, AIHA Publications, American Industrial Hygiene Association, Fairfax, Virginia, pp. 37–40.

Flannigan, B., McCabe, E. M., and McGarry, F. (1991). Allergenic and Toxigenic Microorganisms in Houses, *J. Applied Bacteriology* 70:61–73.

Freund, R., and Wilson, W. (1997). *Statistical Methods*, Academic Press, Chestnut Hill, MA, pp. 142–145.

Hunter, C. A., Grant, C., Flannigan, B., and Bravery, A. F. (1988). Mould in buildings: The Air Spora of Domestic Dwellings, *International Biodeterioration* 24:81–101.

Hyvärinen, A., Meklin, T., Reponen, T., Kotimaa, M., and Nevalainen, A. (1996). Indoor Air Fungi during the Heating Season. *The 7th International Conference of Indoor Air Quality and Climate*, Nagoya, Japan, Vol. 2, pp. 1089–1094.

Hyvärinen, A., Reiman, M., Meklin, T., Husman, T., and Nevalainen, A. (1999). Fungal Exposure and IgG-Levels in Houses with and without Mold Problems. In *Proceedings of Third International Conference on Bioaerosols, Fungi and Mycotoxins 1999*, Eastern New York Occupational and Environmental Health, Saratoga Springs, New York, pp. 166–169.

Hyvärinen, A., Reponen, T., Husman, T., Ruuskanen, J., and Nevalainen, A. (1993). Characterizing Mold Problem Buildings: Concentrations and Flora of Viable Fungi, *Indoor Air* 3:337–343.

Lehtonen, M., Reponen, T., and Nevalainen, A. (1993). Everyday Activities and Variation of Fungal Spore Concentrations in Indoor Air, *International Biodeterioration and Biodegradation* 31:25–39.

Levetin, E. (1995). Fungi. In *Bioaerosols*, edited by H. Burge. Lewis Publishers, Ann Arbor, MI, p. 102.

Miller, D. (1992). Fungi as Contaminats in Indoor Air, *Atmos. Environ.* 26A(12):2163–2172.

Ministry of Social Affairs and Health (1997). Sisäilmaohje, *Sosiaali- ja terveysministeriön oppaita* 1, pp. 56–72 (in Finnish).

Nevalainen, A., Pasanen, A.-P., Niininen, M., Reponen, T., Kalliokoski, P., and Jantunen, M. (1991). The Indoor Air Quality in Finnish Homes with Mold Problems, *Environ. Inter.* 17:299–302.

Nevalainen, A., Pastuszka, J., Liebhaber, F., and Willeke, K. (1992). Performance of Bioaerosol Samplers: Collection Characteristics and Sampler Design Considerations, *Atmos. Environ.* 26A(4):531–540.

Pasanen, A.-L., Niininen, M., Kalliokoski, P., Nevalainen, A., and Jantunen, M. (1992). Airborne *Cladosporium* and Other Fungi in Damp versus Reference Residences, *Atmos. Environ.* 26B(1):121–124.

Pasanen, A.-L., Pasanen, P., Jantunen, M. J., and Kalliokoski, P. (1991). Significance of Air Humidity and Air Velocity for Spore Release into the Air, *Atmos. Environ.* 25A:459–462.

Rautiala, S., Reponen, T., Hyvärinen, A., Nevalainen, A., Husman, T., Vehviläine, A., and Kalliokoski, P. (1996). Exposure to Airborne Microbes during the Repair of Moldy Buildings, *Amer. Ind. Hygiene Assoc. J.* 57:279–284.

Reponen, T., Hyvärinen, A., Ruuskanen, J., Raunemaa, T., and Nevalainen, A. (1994). Comparison of Concentrations and Size Distributions of Fungal Spores in Buildings with and without Mould Problems, *J. Aerosol Sci.* 25(8):1595–1603.

Reponen, T., Nevalainen, A., Jantunen, M., Pellikka, M., and Kalliokoski, P. (1992). Normal Range Criteria for Indoor Air Bacteria and Fungal Spores in a Subarctic Climate, *Indoor Air* 2:26–31.

Reponen, T., Nevalainen, A., and Raunemaa, T. (1989). Bioaerosol and Particle Mass Levels and Ventilation in Finnish Homes, *Environ. Inter.* 15:203–208.

Samson, R., Flannigan, B., Flannigan, M., Verhoeff, A., Adan, O., and Hoekstra, E. (1994). Recommendations. In Health Implications of Fungi in Indoor Environments, Air Quality Monographs. vol. 2, Elsevier Science B.V., Amsterdam, pp. 529–538.

Strachan, D. P., Flannigan, B., McCabe, E. M., and McGarry, F. (1990). Quantification of Airborne Moulds in the Homes of Children with and without Wheeze, *Thorax* 45:382–387.

Verhoeff, A., van Wijnen, J., Boleij, J., Brunekreef, B., van Reenen-Hoekstra, E., and Samson, R. (1990). Enumeration and Identification of Airborne Viable Mould Propagules in Houses, *Allergy* 45:275–284.

WHO (1990). *Indoor Air Quality: Biological Contaminants*, Report on WHO meeting, Rautavaara, 1988. WHO Regional Publications, European Series no. 31.

Zoberi, M. H. (1961). Take-off of Mould Spores in Relation to Wind Speed and Humidity, *Annals of Botany* 25:53–64.